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In re: Markham et al.
International Appln. No.: PCT/GB00/00537
International Filing Date: February 18, 2000
For: LATENCY-ASSOCIATED REGULATORY REGION
FROM HERPESVIRUS SAIMIRI HVS

Date: August 17, 2001

Commissioner for Patents
Washington, DC 20231
Attn: DO/EO/US

APPLICATION FILED UNDER 37 CFR 1.41(c)

Sir:

The above-identified application is being filed on behalf of the inventors, **Alexander Fred Markham,** resident of Molecular Medicine Unit, Clinical Sciences Building, St. James's University Hospital, Leeds LS9 7TF, Great Britain; and **Adrian Whitehouse,** resident of Molecular Medicine Unit, Clinical Sciences Building, St. James's University Hospital, Leeds LS9 7TF, Great Britain, under the provisions of 37 CFR 1.41(c). A Declaration and Power of Attorney from the inventors will follow, 37 CFR 1.63.

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LATENCY-ASSOCIATED REGULATORY REGION FROM HERPESVIRUS SAIMIRI (HVS)

The present invention relates to a method of virus manipulation; means therefor and products thereof which have particular, but not exclusive, application in gene therapy/vaccine development.

Background to the Invention

Herpesvirus saimiri (HVS) is a lymphotropic *rhadinovirus* (γ-2 herpesvirus) which causes persistent infection in its natural host the squirrel monkey (*Saimiri sciureus)* without causing any obvious symptoms of disease. HVS has been subdivided into three groups (A, B and C) on the basis of the sequence of the open reading frame of H. saimiri transformation-associated protein (STP) (Fleckenstein & Desrosiers, 1982; Medveczky et al., 1984). The structure of the HVS genome consists of a unique, low G+C content DNA segment (L-DNA) approximately 110kb in length, flanked by multiple tandem repeats of high G+C content DNA (H-DNA) (Albrecht et al., 1992; Bankier et al., 1985). Analysis indicates it shares limited homology with other herpesviruses. Examples of such herpesviruses include Epstein Barr Virus (EBV), bovine herpesvirus 4 and murine gammaherpesvirus 68 (MHV68) (Blubot et al., 1992; 1996; Virgin et al., 1997). The genomes of EBV, BHV, MHV68 and HVS have been shown to be generally co-linear, in that homologous sequences are found in approximately equivalent locations and in the same relative orientation. However, conserved gene blocks are separated by unique genes with respect to each virus (Virgin et al., 1997). Genes which are expressed in HVS in the latent state are currently unknown.

HVS has a number of features which make it an attractive candidate for use as a gene delivery vector. These include the potential to package and deliver in excess of 50kb of heterologous DNA, the ability to infect non-dividing cells and the maintenance of the viral genome as a stable episome in a latently infected host cell. The ability of herpes viruses to adopt a latent state in infected cells is a particularly attractive

feature in terms of their use as gene delivery vehicles. In addition, because HVS is a non-human pathogen, it should not elicit a primary immune response on introduction into a human host. Primary immune response is a fundamental problem associated with human herpesvirus gene delivery systems which reduces the efficency of these vectors.

In our studies, we generated a recombinant HVS based on the non-transforming strain A11, which expresses the green fluorescent protein (GFP) gene (Whitehouse et al., 1998b). This virus contains the GFP gene under the control of the constitutive human cytomegalovirus (HCMV) early promoter inserted into the rightmost flanking region of H-DNA. We have demonstrated that this recombinant HVS-GFP was able to infect a wide range of human cancer cell lines, including T-cell (Jurkat), pancreatic (MIAPACA), colorectal (SW480) and lung carcinoma cells (A549). Thus, we have continued investigation of this recombinant HVS as we believe it to be an ideal candidate as a gene delivery vector.

The use of an efficient promoter which can drive stable long term expression of a transgene is a prerequisite for the development of any gene delivery vector. A variety of promoters have been utilised in herpes simplex virus (HSV) vectors including neuronal-specific promoters such as the neurone-specific enolase promoter, the neurofilament promoter and tyrosine hydroxylase promoter, as well as viral promoters such as the HSV thymidine kinase promoter and the HCMV immediate early promoter. Studies showed, however, that these promoters are unsuitable for long term expression *in vivo*, due to promoter silencing effects (Fink et al., 1996; Glorioso et al., 1992;1995). There is a need, therefore, to identify viral regulatory regions which can be used to drive stable long term expression of a transgene.

Recently, recombinant HSV-1 viruses have been produced in which expression of the *lacZ* and *lacZ-neo* cassettes are driven by the latency-associated-transcript (LAT) promoter (Lachmann & Efstathiou, 1997). Peripheral infection of neurones with these viruses results in stable long-term expression of a β-galactosidase transgene for

at least 190 days post-infection. Therefore, we believe that it would be advantageous to identify and characterise HVS regulatory regions associated with latency, if they exist, to drive long term stable expression of heterologous transgenes for the future development of HVS as a gene delivery system. In the course of our investigations to identify viral regulatory regions which can be used to drive stable long term expression of a transgene, we serendipitously identified a cluster of HVS genes which are apparently expressed specifically in the latent state and we provide evidence to this effect. The DNA sequence which unexpectedly drives expression of this series of transcripts has been identified. This sequence provides the advantages as a promoter to drive therapeutic gene expression discussed above.

In this application, we describe the identification of a cluster of genes encoding ORF71-73 which are latently expressed in an A549 cell line stably transduced by HVS-GFP. We have characterised a region of 2000 bp immediately upstream of the coding sequence of ORF73 and demonstrated that this regulatory region, when transfected into a human 293T cell line, is able to drive active expression of the GFP reporter gene. This result demonstrates that the upstream region of ORF73 contains regulatory sequences which may be utilized to drive expression of heterologous transgenes in a range of human cell lines. Therefore we believe that the ORF73 promoter, which drives virus-encoded gene expression whilst the HSV is present in a cell in a latent state, is an ideal choice of regulatory sequence for driving stable long term expression of a transgene in HVS-based gene delivery vectors.

Furthermore, in order to further investigate the possibility of using the ORF73 regulatory region as a promoter to drive long term expression of a heterologous transgene, a number of PCR fragments containing sequence immediately upstream of the ORF73 initiation codon were amplified by PCR and cloned into a reporter plasmid containing the GFP gene. These reporter constructs were transfected into the human 293T cell line and we have demonstrated that some of these fragments contain a regulatory region sufficient to drive heterologous gene expression in a human 293T cell line.

We believe that *Herpesvirus saimiri* (HVS) is an attractive candidate for use as a gene therapy vector as it has the ability to enter a latent mode of infection in which the viral genome is maintained as a stable episome in the host cell. We have generated a recombinant HVS in which the gene encoding green fluorescent protein (GFP) is expressed under the control of the constitutive human cytomegalovirus (CMV) promoter (HVS-GFP). This recombinant virus is able to stably transduce a range of human cell lines including the lung carcinoma cell line, A549, and direct production of GFP. However, it is known that the human CMV promoter is not effective in many circumstances for sustaining transgene expression in gene therapy *in vivo*. We have therefore sought to identify promoters which might be functional during latent infection with the HVS vectors.

Statement of the Invention

In the broadest aspect of the invention there is provided a gene delivery system/vaccine comprising a promoter which functions in a vector gene delivery system/vaccine during periods when the gene therapy vector is present in the cell in a latent state. The present invention is capable of regulating long term gene expression in the gene delivery system/vaccine and is capable of controling the expression of transgenes in a range of human or animal cells.

According to a first aspect of the invention there is provided a nucleic acid comprising a nucleic acid sequence which encodes a promoter and which hybridises under high stringency conditions to the nucleic acid sequence of SEQ ID NO:1, fragments and/or variants thereof, for use in gene therapy.

Preferably, hybridisation occurs under stringent conditions such as 1 x SSC, 0.1% SDS at 65°C.

Preferably, said promoter comprises a nucleic acid sequence of at least 329 bp and up to 2000 bp, more preferably said promoter comprises a nucleic acid sequence of up to

a length of 329, 630, 1000 or 1500 bp or any other selected fragment or variant thereof. It will be appreciated that it is possible that the promoter sequence of the invention may be less than 329 bp so long as the effective sequence encoding the promoter is included in the invention.

5

According to a second aspect of the invention there is provided a recombinant DNA molecule containing at least one insert comprising the nucleic acid sequence of SEQ ID NO:1, fragment or variant thereof, encoding a promoter.

10 Thus it will be appreciated that the invention includes nucleic acids comprising (i) a sequence of up to 2000bp which encodes the promoter, (ii) fragments of selected bp lengths within the sequence and (iii) variants thereof, as well as recombinant DNA molecules containing insert(s) of the promoter sequence therein.

15 According to a third aspect of the invention there is provided a gene therapy system comprising a vector which includes a nucleic acid sequence selected from the group consisting of the nucleic acid sequence of SEQ ID NO :1, and fragments and variants thereof as well as nucleic acid sequences which hybridise under high stringency conditions to the sequence of SEQ ID NO :1, or a part thereof, wherein said system is
20 capable of driving heterologous gene expression during periods of latent infection by the vector in a target cell population.

Preferably, the gene therapy system further includes any one or more of the features herein before described.

25

Preferably, said vector additionally comprises at least one therapeutic nucleic acid, whereby the promoter encoded by SEQ ID NO:1 or fragment or variant thereof acts to drive expression of said the at least one therapeutic nucleic acid.

30 Reference herein to therapeutic nucleic acid is intended to include a therapeutic gene or fragment or variant thereof.

The vector of said gene delivery system may be viral or non-viral.

Preferably, said gene therapy system is capable of long term gene expression.

Reference herein to long term gene expression includes gene expression for at least several hours and optimally at least several months, for example and without limitation, from 2 hours to six months or more.

According to a fourth aspect of the invention there is provided use of a gene therapy system as herein before described for long term gene expression.

It will be appreciated by those skilled in the art that the invention comprises a gene therapy system and that, in preferred embodiments the vector may be either viral or non-viral. The expression of a therapeutic gene can be regulated by a promoter, typically of up at least 329 bp and up to 2000 bp, the system being capable of driving heterologous gene expression during periods of latent infection of a target cell population. Thus, foreign transgenes can be controlled by, for example, a natural promoter, which is active in the latent mode of viral infection. The specifics of the gene expression and the nature of the vector is not intended to limit the scope of the application.

According to a fifth aspect of the invention there is provided an HVS comprising a nucleic acid sequence encoding a promoter of SEQ ID NO:1, or fragment or variant thereof or a nucleic acid sequence which hybridises under high stringency conditions to the sequence of SEQ ID NO:1, fragment or variant thereof, which promoter acts in the latent state, the sequence encoding for the promoter being positioned so as to drive expression of at least one therapeutic nucleic acid which has been inserted in the HVS.

The preferred embodiments of the fifth aspect of the invention include those listed in accordance with the aforementioned first and third aspects of the invention.

Preferably, the HVS of the present invention may be rendered ineffective and its activity terminated by the appropriate co-administration of an anti-herpetic pharmaceutical such as acyclovir.

According to a sixth aspect of the invention there is provided a method of manufacturing an expression vector comprising the promoter of the first aspect of the invention or the gene therapy system of the third aspect of the invention or the HVS vector of the fifth aspect of the invention, the method comprising transfecting a cell with a nucleic acid sequence encoding said promoter of SEQ ID NO:1, or fragment or variant thereof or a nucleic acid sequence which hybridises under high stringency conditions to the sequence of SEQ ID NO:1 or any part thereof.

The invention includes methods which comprise selecting the promoter and amplifying it and subsequently purifying it prior to transfecting a cell population, preferably a selected target cell population.

According to a seventh aspect of the invention there is provided a method of treatment comprising administering a therapeutically effective amount of the promoter of the first aspect of the invention or a gene therapy system of the third aspect of the invention or a HVS gene therapy vector of the fifth aspect of the invention, to an individual requiring treatment.

According to an eighth aspect there is provided the promoter of the first aspect of the invention or the gene therapy system of the third aspect of the invention or the HVS vector of the fifth aspect of the invention for use as a pharmaceutical.

According to a yet further aspect of the invention there is provided a pharmaceutical composition comprising the promoter of the first aspect of the invention or a gene therapy system of the third aspect of the invention or a HVS gene therapy vector of the fifth aspect of the invention,the pharmaceutical additionally comprises a pharmaceutically acceptable excipient, diluent or carrier and ideally said

pharmaceutical can be formulated as a nasal spray, or for injection or for oral/paraenteral administration into a individual requiring treatment.

According to a yet further aspect of the invention there is provided use of the promoter of the first aspect of the invention or the gene therapy system of the third aspect of the invention or the HVS vector of the fifth aspect of the invention in the manufacture of a medicament for treating cancer.

According to a yet further aspect of the invention there is provided use of the promoter of the first aspect of the invention or the gene therapy system of the third aspect of the invention or the HVS vector of the fifth aspect of the invention in the manufacture of a medicament for treating degenerative disorders.

According to a yet further aspect of the present invention is an isolated nucleic acid encoding a promoter, the nucleic acid may be selected from the group consisting of:

(a) DNA having the nucleotide sequence given herein as SEQ ID NO:1 and which encodes the promoter;

(b) nucleic acids which hybridize to DNA of (a) above (e.g., under stringent conditions) and which encode the promoter ; and

DNAs of the present invention include those of closely related sequences to, and having essentially the same biological properties as, the promoter disclosed herein, and particularly the DNA disclosed herein as SEQ ID NO:1. This definition is intended to encompass natural allelic variations therein. Thus, DNAs which hybridize to DNA disclosed herein as SEQ ID NO:1 (or fragments or derivatives thereof which serve as hybridization probes as discussed below) and which encode the promoter of the present invention are to be included in the definition.

Conditions which will permit other DNAs which encode the promoter of the present invention and hybridize to the DNA of SEQ ID NO:1 disclosed herein can be determined in accordance with known techniques. For example, hybridization of

such sequences may be carried out under conditions of reduced stringency, medium stringency or even stringent conditions (e.g., conditions represented by a wash stringency of 35-40% Formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 37°C; conditions represented by a wash stringency of 40-45% Formamide with 5x Denhardt's solution, 0.5% SDS, and 1x SSPE at 42°C; and conditions represented by a wash stringency of 50% Formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 42°C, respectively) to DNA of SEQ ID NO:1 disclosed herein in a standard hybridization assay. See, e.g., J. Sambrook et al., *Molecular Cloning, A Laboratory Manual* (2d Ed. 1989) (Cold Spring Harbor Laboratory). In general, sequences which code the promoter of the present invention and which hybridize to the DNA of SEQ ID NO:1 disclosed herein will be at least 75% homologous, 85% homologous, and even 95% homologous or more with SEQ ID NO:1.

The invention will now be described with reference to the following Figures wherein:

Figure 1 represents SEQ ID NO:1;

Figure. 2 illustrates expression of GFP in A549 cells from episomal HSV-GFP;

Figure. 3 illustrates a schematic representation of the map positions of restriction fragments of the HVS genome;

Figure 4 illustrates untreated or latency infected A549 cell total RNA separated on 1% denaturing agarose gels, Northern blotted and hybridized with the labelled (a) *Eco* D fragment and (b) with the labelled *Eco* J fragment, respectively of HVS genomic DNA;

Figure 5 illustrates untreated or latency infected A549 cell total RNA separated on 1% denaturing agarose gels, gel electrophoresis, Northern blotted and hybridized

with (a)the labelled *Eco* C fragment and (b) with the labelled *KpnE* fragment, respectively of HVS genomic DNA;

Figure 6 illustrates untreated or latency infected A549 cell total RNA separated on 1% denaturing agarose gels, Northern blotted and hybridized with a) ORF71 b) ORF72 c) ORF73;

Fig.7 illustrates expression of GFP in transfected human 293T cell lines, and

Table 1 represents results obtained by Northern blot analysis.

Brief Description of the Figures

Fig.1. (SEQ ID NO:1) sequence of the 2000bp promoter.

Fig. 2. Expression of GFP in A549 cells stably transduced with a recombinant HVS

Fig. 3 A schematic representation of the map positions of restriction fragments resulting from digestion of the HVS genome with either *Eco*RI or *Kpn* I. The fragments represent the entire L-DNA region (112kb) of *H. saimiri.*

Fig. 4 Hybridization with the labelled *Eco* D fragment (a) and the labelled *Eco* J fragment (b). Each lane was loaded with 6μg of total RNA and analyzed by Northern blotting and hybridization with the labelled probe. Lane 1 contains extract from uninfected A549 cells; lane 2 - A549 cells stably transduced with the recombinant HVS-GFP virus and cultured in the presence of G418 (0.6mg/ml); lane 3 - A549 cells stably transduced with the recombinant HVS-GFP virus and cultured in the absence of G418; lane 4 - uninfected OMK cells; lane 5 - RNA extracted from OMK cells 8 hours after infection with the recombinant HVS-GFP virus; lane 6 - RNA extracted from OMK cells 16 hours after infection with the recombinant HVS-GFP virus; lane 7 - RNA extracted from OMK cells 24 hours after infection with the recombinant HVS-GFP virus; lane 8 - RNA extracted from OMK cells 48 hours after infection

with the recombinant HVS-GFP virus. Hybridization with an *Actin* probe as a control for amounts of RNA loaded, is shown below.

Fig. 5 Hybridization with the labelled *Eco* C fragment (a) and *Kpn* E fragment (b). Each lane was loaded with 6μg of total RNA and analyzed by Northern blotting and hybridization with the labelled probe. Lane 1 contains extract from uninfected A549 cells; lane 2 - A549 cells stably transduced with the recombinant HVS-GFP virus and cultured in the presence of G418 (0.6mg/ml); lane 3 - A549 cells stably transduced with the recombinant HVS-GFP virus and cultured in the absence of G418; lane 4 - uninfected OMK cells; lane 5 - RNA extracted from OMK cells 8 hours after infection with the recombinant HVS-GFP virus; lane 6 - RNA extracted from OMK cells 16 hours after infection with the recombinant HVS-GFP virus; lane 7 - RNA extracted from OMK cells 24 hours after infection with the recombinant HVS-GFP virus; lane 8 - RNA extracted from OMK cells 48 hours after infection with the recombinant HVS-GFP virus. Again, hybridisation with an *Actin* probe was used as the control for RNA loading, as shown below.

Fig.6 Hybridization with a) ORF71 b) ORF72 c) ORF73. Each lane was loaded with 6μg of total RNA and analyzed by Northern blotting and hybridization with the labelled probe. Lane 1 contains extract from uninfected A549 cells; lane 2 - A549 cells stably transduced with the recombinant HVS-GFP virus and cultured in the presence of G418 (0.6mg/ml); lane 3 - A549 cells stably transduced with the recombinant HVS-GFP virus and cultured in the absence of G418; lane 4 - uninfected OMK cells; lane 5 - RNA extracted from OMK cells 8 hours after infection with the recombinant HVS-GFP virus; lane 6 - RNA extracted from OMK cells 16 hours after infection with the recombinant HVS-GFP virus; lane 7 - RNA extracted from OMK cells 24 hours after infection with the recombinant HVS-GFP virus; lane 8 - RNA extracted from OMK cells 48 hours after infection with the recombinant HVS-GFP virus. *Actin* probe controls are again shown below.

Fig.7 Expression of GFP in human 293T cell lines. Cells were grown to approximately 70% confluence and transfected with 2μg of the reporter plasmids p73.1-4-GFP using Lipofectamine according to the protocol described by the manufacturer, Life Technologies.

Materials and Methods

Viruses, cell cultures and transfections

Recombinant HVS (Strain A11) was propagated in Owl Monkey Kidney (OMK) cells which were maintained in Dulbecco's modified Eagle medium (Life Technologies) supplemented with 10% foetal calf serum. Human lung carcinoma A549 and 293T cells were maintained in Dulbecco's modified Eagle medium (Life Technologies) supplemented with 10% foetal calf serum. Jurkat cells were maintained in RPMI (Life Technologies) supplemented with 5% foetal calf serum.

For transfection, cells were seeded at approximately $5x10^5$ cells per 35 mm diameter Petri dish 24 h prior to transfection. Plasmids used in the transfections were prepared using the Qiagen Plasmid kits according to the manufacturer's directions. Transfections were performed using Lipofectamine™ (Gibco BRL) as described by the manufacturer using 2 μg of the appropriate plasmid.

Total RNA Extraction

Cells were lysed using Trizol reagent (Life Technologies). Chloroform (0.2ml) was then added and the solution vortex-mixed for 15s and stored at room temperature for 5 min. Samples were centrifuged for 15 min at 4°C, and the aqueous phase containing nucleic acids was precipitated using 0.5ml of isopropanol. The pellet was washed with 70% ethanol, resuspended in 20μl DEPC-treated water (0.1% solution) and stored at -70°C.

Northern Blot Analysis

Northern blot analysis was performed essentially as described by Sambrook et al. (1989). Total RNA was isolated from untreated or HVS-transduced A549 cells or from lytically infected OMK cells at 8, 16, 24 and 48 hours post infection, and separated by electrophoresis on 1% denaturing formaldehyde agarose gels. The RNA was transferred to Hybond-N membranes and hybridised with radiolabelled ^{32}P-labelled random primed probes made from restriction fragments derived from the HVS genome (Figure 3). Hybridisations were performed for 12 hours at 65°C using ExpressHyb™ buffer (Clontech).

HVS Genomic Probes

The HVS genome can be cleaved with *Eco*RI and the resultant fragments cloned into the plasmid vector pACYC184 or *Kpn* I fragments can be cloned into the vectors pJC81 or pWD7 (Knust et al., 1983). These genomic fragments were excised from the vectors by digestion with either *Eco*RI or *Kpn* I. The ORF71 gene was amplified by PCR using the primer pair; ORF71F (SEQ ID NO:2) dCGC GGA TCC GGC AAG GTC ACT TCG CCC TAT CTG-3', ORF71R (SEQ ID NO:3) 5'dCCG GAA TTC CTG TGT TAC ACA TAA CAG ACT-3'. The ORF72 gene was amplified using the primer pair; ORF72F (SEQ ID NO:4) 5'dCGC GGA TCC GCT GCA ATG GCA GAT TCA CC-3'; ORF72R (SEQ ID NO:5) 5'dCCG GAA TTC GGT CTG CAG TTA GTG TTG TCA G-3'. The ORF73 gene was amplified using the primer pair ORF73F (SEQ ID NO:6) 5'dACG CGT CGA CCC ATC TAT AAT TGC AAC AAA CAC C-3'; ORF73R (SEQ ID NO:7) 5'd-CCC AAG CTT CAC ATA TAT GAA TGC TAG TGC AC-3'. The PCR (1 cycle of 5 min at 95°C; 30 cycles of 1min at 95°C, 1 min at 55°C, 1 min at 72°C; 1 cycle of 10 min at 72°C) was performed using 2U of Klentaq DNA Polymerase (Clontech). Probes were radio-labelled using the Megaprime kit according to the method described by the manufacturer (Amersham).

Plasmids

In order to make the reporter constructs p73.1-4GFP, 632, 1000, 1500 and 2000 bp sequences immediately upstream of the ORF73 initiation codon (Figure 1) were

amplified by PCR (1 cycle for 5 min at 95°C; 35 cycles 1 min at 95°C, 1 min at 55°C, 1 min at 72°C; one cycle for 10 min at 72°C). For p73.1GFP, the primer pair (SEQ ID NO:8) 5'dACG CGT CGA CCC ATC TAT AAT TGC AAC AAA CAC G-3'; (SEQ ID NO:9) 5'dCCC AAG CCT CAC ATA TAT GAA TGC TAG TGC AC-3' were utilised. These primers incorporated terminal *Hin*dIII and *Sal* I restriction sites respectively, for convenient cloning of the PCR product. In order to amplify p73.2-4 the forward primers (SEQ ID NO:10) 5'dGCA CTG CAG CAC CAT CAC ATG AGG AGG TGC-3'; (SEQ ID NO:11) 5'dGCA CTG CAG CCA TGC AGC AGC CAT GCG CTG CC-3' and (SEQ ID NO:12) 5'd-GCA CTG CAG CCC AGA GAG CTG GAC ACT AG-3' and the same reverse primer (SEQ ID NO:13) 5'dCGC GGA TCC CCA TCT ATA ATT GCA ACA AAC ACG-3' were used. These primers contained the restriction sites *Pst*I and *Bam*HI, respectively for convenient cloning of the PCR products. Upon digestion with the appropriate restriction enzymes, the PCR products were cloned into the reporter plasmid pEGFP (Clontech) to derive the expression constructs p73.1-4-GFP, respectively.

Results

Production of stably transduced A549 cell lines with HVS-GFP.

In order to identify any HVS latency-associated transcripts which might exist, A549s cells were infected with HVS-GFP and cultured in the presence of G-418. After 48 hours approximately 75% of the cells were found to express the transgene and this increased to 100% by day 12. Fluorescence microscopy confirmed that the GFP protein was expressed in these cells (Fig.2.a) and Southern blot analysis on viral DNA confirmed that the viral genome was maintained episomally (Fig 2.b). This cell line formed the basis of the HVS-latency model as the cells remained green, expressing the transgene for 6 months, demonstrating that HVS was stably maintained in a latent episome form (Fig 2.b).

Transcription mapping of the HVS genome in latently infected A549 cells

In order to identify which genes were expressed in the latent episomal state of HVS, Northern blot analysis was performed. Total RNA was extracted from an A549 cell line which had been stably transduced with a recombinant HVS. As controls, total RNA was extracted from a lytic infection of OMK cells at 8, 16, 24 and 48 h.p.i. and from uninfected A549 cells. Northern blots were hybridized with restriction fragments of genomic HVS DNA which spanned the complete coding region of the HVS genome between the two flanking regions of H-DNA. The location of these restriction fragments on the HVS genome is shown in Fig.3.

Rather than present each Northern blot individually we have chosen to summarize the results of the analysis in Table 1. Only a semi-quantitative estimate of signal intensity has been made, since this depends upon a number of factors including probe length, exposure time, transcript length and the specific activity of the probe. Also, since the aim of this work was simply to identify which genes are transcribed in a latent HVS infection we did not consider it necessary to accurately quantify the intensity of each signal but rather to make a qualitative assessment by comparing signal intensity in A549 cells with that in lytically infected OMK cells.

High levels of gene expression were observed in control lytic OMK cell infections when hybridized with fragments *Eco* D and *Eco* J (Figure 4). The *Eco* D fragment contains the ORF50 gene, the product of which is a strong transcriptional activator responsible for initiating expression of delayed early (DE) and late viral genes in the lytic cascade. Similarly, the *Eco* J fragment contains the ORF57 gene, which is activated by ORF50 and also activates expression of DE and late viral genes (Whitehouse et al;1997b;1998a;b). In comparison with the levels of gene expression observed in the lytically infected OMK cells, negligible levels of gene expression were detected in the stably transduced A549 cells when probed with *Eco* D or *Eco* J. Some low level of lytic gene expression may be due to a very low background of lytic replication occurring in a sub-population of the A549 cells infected with the recombinant HVS. High levels of gene expression were also observed in the lytically

infected OMK cells when probed with genomic fragments containing viral DE or late genes but again, negligible gene expression was detected in the stably transduced A549 cells when probed with the same fragments. Fig. 4 shows Northern blots using probes made from the *Eco* D and *Eco* J fragments. However, incubation of Northern blots with probes specific for either the *Eco* C or the *Kpn* E fragment detected comparable levels of a transcript approximately 6kb in length in transduced A549 cells as well as lytically infected OMK cells (Fig. 5). Both the *Eco* C and *Kpn* E genomic fragments unexpectedly share a region of overlap which contains ORFs 71-73.

ORFs71-73 are expressed in A549 cells stably transduced with recombinant HVS

In order to further investigate the pattern of gene expression observed in the stably transduced A549 cells, Northern blot analysis was performed using specific probes for ORF 71-73. The results are shown in Fig. 6. Hybridization with each of the three probes detected two transcripts of approximately 6kb and 4.4kb in both stably transduced A549 cells and lytically infected OMK cells. The expression levels of these transcripts in the stably transduced A549 cells are comparable with levels in the lytically infected cells and are very significantly higher than the very low levels of expression detected in these cells when hybridizing with probes containing other genes expressed in the lytic mode of infection, strongly suggesting that unexpectedly these transcripts are expressed in the latent episomal state.

The region immediately upstream of ORF73 contains a promoter which is active in the latent state and which is able to drive expression of a transgene in 293T cells.

The development of HVS as an effective gene delivery vector requires the use of promoters which can drive stable long term expression of heterologous transgenes. Current promoters such as the constitutive HCMV promoter, which have been used to drive expression of GFP in our recombinant HVS, are susceptible to silencing effects which are poorly understood. A viral promoter driving expression of genes active in the latent state would be an ideal candidate for use in regulating long term expression of a foreign transgene. Having identified the active expression of ORF71-73 in cells containing episomally maintained HVS in the latent state, we investigated

whether the regulatory region upstream of ORF73 could be utilized to drive expression of a transgene.

A number of PCR fragments encompassing 630, 1000, 1500 and 2000 bp of sequence immediately upstream of the initiation codon of ORF 73 were amplified by PCR. The primers used in the PCR were designed so that the final products contained *Hind*III and *Sal* I restriction sites at their 5' and 3' termini, respectively. Each PCR product was purified and cloned into the polylinker site of the plasmid, pEGFP to generate the reporter plasmids, p73.1-4GFP, respectively. The reporter plasmids were each transfected into the human 293T cell line and GFP expression analysed by fluorescence microscopy 48 hours post transfection. Results are shown in Figure 7. All four fragments containing the upstream sequence of ORF 73 are sufficient to drive heterologous gene expression in human 293T cells.

The ability of *Herpesvirus saimiri* to enter a latent mode of infection in a human cell in which the viral genome is maintained as a stable episome makes this virus an attractive candidate for use as a gene delivery vector. Previously we have described a recombinant HVS containing an expression cassette in which the GFP gene is under the control of the constitutive HCMV promoter. We have shown that this virus is able to stably transduce a range of human cancer cell lines, including the lung carcinoma line, A549. In this cell line the viral genome is maintained as a stable episome and the GFP gene product is produced, demonstrating that HVS can be used as a vector to deliver foreign genes into tumour cells.

Despite some expression of GFP, however, the HCMV promoter is not an ideal choice for driving long term stable expression of a heterologous transgene because it is susceptible to poorly understood silencing effects which reduce the activity of the promoter.

A viral promoter which is active in the latent, non-replicative mode of HVS infection would be the ideal choice for driving stable long term gene expression in an HVS-

based gene therapy vector. Identification of genes transcribed in a latent HVS infection would help to identify candidate promoters suitable for use in driving transgene expression in an HVS-based gene delivery vector. We therefore extracted RNA from A549 cells stably transduced with the recombinant HVS-GFP virus and probed with a series of fragments which span the entire coding region of the HVS genome. Hybridization with fragments containing genes encoding immediate early transactivators of the lytic transcriptional cascade detected high levels of gene expression in a lytic infection of OMK cells with the HVS-GFP virus. Similarly, hybridization with fragments containing late genes encoding structural components also detected high levels of gene expression in a lytic infection of OMK cells. In comparison to the lytically infected OMK cells, negligible levels of lytic gene transcription were detected in the stably transduced A549 cells. This may be explained by considering the A549 cells as consisting of two populations of cells, one large sub-population in which the HVS-GFP virus enters a truly latent mode of infection, and a much smaller sub-population of cells in which the HVS-GFP virus may enter a lytic mode of infection. Virus recovery assays from this stably transduced A549 cell line show that a very low level of viral replication does occur.

Hybridization with two specific fragments, *Eco* C and *Kpn* E, detected high levels of gene expression in the stably transduced A549 cells comparable with those in infected OMK cells, a permissive cell line. Such a high level of gene expression could not be due to the very low level of lytic replication in A549 cells, since expression of other lytic cycle genes was so low as to be barely detectable in this cell line. A more likely explanation is that the fragments *Eco* C and *Kpn* E hybridize to mRNA from genes which are expressed when HVS is in a latent, non-replicative mode of infection as in the majority of the A549 cells.

Analysis of the *Eco* C and *Kpn* E fragments revealed that they both contained ORF71-73. Unexpectedly, hybridization of Northern blots with PCR products of ORF71, 72 and 73 detected high levels of two transcripts in both our stably transduced A549 cell line and lytically infected OMK. In each experiment,

hybridization with ORF71-73 detected the same transcripts, 6kb and 4.4kb in length, suggesting that these genes are transcribed as a polycistronic mRNA from the ORF73 promoter, since this gene lies at the rightmost end of the cluster.

From these studies we conclude that a region including ORF71-73 is expressed in both the lytic and latent modes of HVS infection. Furthermore we believe the regulatory region which expresses the latent transcript is an ideal choice for driving stable long term expression of a transgene. We therefore investigated whether the ORF73 promoter was active in a range of human cell lines to demonstrate whether it could be used to drive a heterologous transgene. A commonly encountered problem with currently used strong promoters such as the HCMV promoter is that their activity is reduced by a silencing effect in a number of cell lines. We speculated that the regulatory region of a latently expressed HVS gene should not be susceptible to this effect and would be an ideal choice for driving expression of foreign genes.

Similar problems with silencing have been encountered in Herpes simplex virus (HSV) based vectors. In a latent infection of neurons by HSV, the virus genome is maintained in a nonlinear, episomal, nucleosome bound state and transcription is restricted to a single region encoding two highly abundant, polyadenylated latency associated transcripts (LATs) (Fraser et al., 1992; Stevens et al, 1987). The TATA box and basal transcriptional elements which constitute the LAT promoter reside within an approximately 700bp region upstream of the 2kb major LAT (Dobson et al., 1995). This core LAT promoter is not sufficient to drive prolonged reporter gene expression during latency however, as regulatory elements found within the first transcribed 1.5kb LAT sequences have also been found to be necessary for full promoter activity (Lokensgard et al., 1994; Perng et al, 1996). The majority of heterologous promoters used in HSV-based vectors have all resulted in either transient or low-level, long-term gene expression in only a small proportion of transduced cells (Bloom et al.,1995; Ecob-Prince et al., 1995; Lachmann et al., 1996). Recently, however, the upstream and downstream elements of the LAT promoter have been used to drive expression of *lacZ* and *lacZ-neo* reporter genes in a recombinant HSV-1. After peripheral infection this recombinant HSV was capable of

driving stable, long-term expression of β-galactosidase in the peripheral nervous system of mice for at least 190 days postinfection (Lachmann et al., 1997).

In order to determine whether regulatory sequences governing the expression of ORF73 could be used similarly to drive foreign gene expression we constructed reporter plasmids, p73.1-4GFP, in which the GFP gene was placed under the control of the various PCR fragments encoding sequences immediately upstream of the ORF 73 gene. Transfection of the reporter constructs into 293T cells showed that GFP was expressed at high levels, indicating that the minimal functional ORF73 promoter was contained within the 632bp upstream of ORF73 and that this promoter could drive expression of a heterologous transgene in a human cell line.

In conclusion, we have found three genes, ORF71, 72, and 73 which are expressed by HVS when maintained as a stable, non-replicating episome in human A549 lung cancer cells. We have also shown that the upstream regulatory sequences of the ORF73 coding region are sufficient to drive expression of a foreign transgene in a human 293T cell line. We believe that this is of crucial importance to the development of HVS as an effective gene therapy vector since foreign transgenes can now be placed under the control of a natural HVS promoter which is active in the latent mode of viral infection.

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Claims

1 A nucleic acid comprising a nucleic acid sequence which encodes a promoter and hybridises under high stringency conditions to the nucleic acid sequence of SEQ ID NO:1, fragments and/or variants thereof.

2. A nucleic acid according to Claim 1 wherein the stringent conditions are 1 x SSC, 0.1% SDS at 65°C.

3. A nucleic acid according to either preceding claim wherein said promoter comprises a nucleic acid sequence of at least 329 bp and up to 2000 bp.

4. A nucleic acid according to Claim 3 wherein said promoter comprises a nucleic acid sequence of up to a length of 329, 630, 1000 or 1500 bp.

5. A recombinant DNA molecule containing at least one insert comprising the nucleic acid sequence of SEQ ID NO:1, or a fragment and/or variant thereof and encoding a promoter.

6. A gene therapy system comprising a vector which includes a nucleic acid comprising a nucleic acid sequence which encodes a promoter and hybridises under high stringency conditions to the nucleic acid sequence of SEQ ID NO:1, fragments and/or variants thereof, wherein said system is capable of driving heterologous gene expression during periods of latent infection by the vector in a target cell population.

7. A gene therapy system according to Claim 6 which further includes any one or more of the features of Claims 2-4.

8. A gene therapy system according to either of Claims 6 or 7 wherein the vector additionally comprises at least one therapeutic nucleic acid, whereby the promoter

encoded by SEQ ID NO:1 or fragment or variant thereof acts to drive expression of the therapeutic nucleic acid.

9. A gene therapy system according to any of Claims 6-8 wherein the vector is viral or non-viral.

10. Use of a gene therapy system according to any of Claims 6-9 for long term gene expression.

11. An HVS including a nucleic acid comprising a nucleic acid sequence which encodes a promoter and hybridises under high stringency conditions to the nucleic acid sequence of SEQ ID NO:1, fragments and/or variants thereof, the promoter acting in the latent state and the sequence encoding the promoter being positioned so as to drive expression of at least one therapeutic nucleic acid which has been inserted in the HVS.

12. An HVS according to Claim 11 and further including any of the features of Claims 2-4, 8 or 10.

13. A method of manufacturing an expression vector comprising a promoter according to any of Claims 1-4, or a gene therapy system according to any of Claims 6-10 or a HVS vector according to either Claim 11 or 12, the method comprising transfecting a cell with a nucleic acid sequence encoding the promoter of SEQ ID NO:1, or fragment or variant thereof or a nucleic acid sequence which hybridises under high stringency conditions to the sequence of SEQ ID NO:1 or any part thereof.

14. A method according to Claim 13 which further comprises the steps of selecting siad nucleic acid sequence and amplifying it and subsequently purifying it prior to transfecting a cell population.

15. A method of treatment comprising administering a therapeutically effective amount of a promoter according to any of Claims 1-4 or a gene therapy system according to any of Claims 6-10 or a HVS vector according to either Claim 11 or 12, to an individual requiring treatment.

16. A promoter according to any of Claims 1-4 or a gene therapy system according to any of Claims 6-10 or a HVS vector according to either Claim 11 or 12, for use as a pharmaceutical.

17. A pharmaceutical composition comprising a promoter according to any of Claims 1-4 or a gene therapy system according to any of Claims 6-10 or a HVS vector according to either Claim 11 or 12, and a pharmaceutically acceptable excipient, diluent or carrier.

18. A pharmaceutical composition according to Claim 17 that is formulated as a nasal spray, or for injection or for oral/parenteral administration.

19. Use of a promoter according to any of Claims 1-4 or a gene therapy system according to any of Claims 6-10 or a HVS vector according to either Claim 11 or 12, in the manufacture of a medicament for treating cancer.

20. Use of a promoter according to any of Claims 1-4 or a gene therapy system according to any of Claims 6-10 or a HVS vector according to either Claim 11 or 12, in the manufacture of a medicament for treating degenerative disorders.

21. An isolated nucleic acid encoding a promoter, the nucleic acid being selected from the group consisting of:

(a) DNA having the nucleotide sequence given herein as SEQ ID NO:1 and which encodes the promoter; and

(b) nucleic acids which hybridize to DNA of (a) above (e.g., under stringent conditions) and which encode the promoter.

Latency-associated regulatory region
(Initiation codon of ORF 73 shown in bold)

FIGURE 1 : SEQ ID NO: 1

5'ACCCAGAGAGCTGGACACTAGAACTAGAACCTAATGCATCAAAGCATTATGAATC
TTTATGGCTCAACTTTCACGTTCCTCAAACTACTAAAAGCATTATATTACAAGCCCT
TCGTGGCACAATTTTCCAGGATGGCTTGTGGCAAGTACTTGGACTGAGATACAAACA
CGATGCTCAAGAATATATTATGCAACAAAATGGAACAATTGCAATGAGTTATCATAG
TGCTAAGATAAATCCTTACTTGTATGCAATGCATTATCCAAGGAACCCCTCTGGCAA
TTCATCTGTAGCTGGCATATGTTCAAAGAATGGCAGGCATCTTGCGTTGCTTGTAGA
ACCAGCCCTTTCTTTTCATACTTGGCAATGGCAACATATACCTAAACCTCTAGTAAC
TTCTCCATGGGCATTAATGTATCAATGTATGTTCTTGTGGTGTGTAAAAGAATGATT
GTACTAAGGAACAGTAATAAAAACTCTGACACTAAGATACGATAATATAACTATTTA
TTTATCAAGTGAGCCGCTCTACACTCTAACAGTGACAAATAGTTTTACACCATGCAG
CCATGCGCTGCCTAAAGAGACTTCCAAACATAGCAAACATCAGAGGTAACATACAAT
AATATAGTACCAACAGCATATATGTACATTGAATTCCATACACTATAGCAGATCTCT
TTGCACATGTCTCTTCTATTACACCAACACGCAACAAAGTATCAATGCTTTCCATAA
TATAGTATGGTATACAAAACACTATGAATAGCAGTGTTGTCATTGTAATTATCGTGA
CTACCTCTGCTCTTTTAGACAGCTTTGTCTTGAATAACTTATAACATGACATACTAT
AGCATATTACAGTAATAAAGAGGGGTCCTGCAAAGCTATACCATGTGTGAAAAGTGT
TTAGCTTTGTGCGTAGCTGCTCAGTCAACACACCATCCTCCTCTATGCAAGAAGATG
GTTCATAATATGATGTCACCATCACATGAGGAAGTGCTCCAAAGCAGGCTAATACAA
ATGAACAGCACAGAAATACTTGCCCAATAAGAGTCTTTTCACCCACAGTCTAGTAG
CACAAAATATTAGCAGACAACGCAAGACACTAATAAAACTAATATGAAAGGAGACC
AATAAATGCTGAGATTTAAGAAAAAAGCTTCCAGCTTACACAGCTCAGTATTCATAA
AAATTTCAAACATGCGCAAAAGTCTCATTAGCAGATACCCAGCTAAGAACAAGCTGT
TGAGACAAAATCCCATCATCAAGTAGTCAAAACTTTGAGCTTGAGCTCTATACTTTA
GAAAAGTCCTCAGTACAAGAGAATTCCCAATTGCATTGCATAAAAACATCAACACAT
ATATGAATGCTAGTGCACTCTCTGAAATTAAAAAGTTCACTACACACGGCGCTACAT
CTCCATAATATATGTCTCCACTATAATTGTAAGAATAGTTGCTAAAGTCTTCACTAC
TGAAGTCCAGCTTGACCTCCATAGCGAACTACAAAATAAATTTATATAAATTATTCA
CCCAATAACTTGAAATTTAAAGAATTAGGACAAAAGAATGTATATCCTACCTTTCTT
TGCAGCCTGACAGCAAGCTACTGAAAAAGTTACTTTTTATTTTGTTTTAGTAGCTAG
GTGTGGTTTTACATATGTTTTGTGGCTACACAGTAGATTTAACAAATAGCCACGCCC
CCTACGCTACGTCTAAGGAGGAGCTTAATTCCAAACGAGTGGCGGGATTTCGCTAAA
GTCACTGAAGAACTTGCATCTTAATTCATCCGCGGCTGCAACCTTCAAACAAAAAAG
GAGGTTTCGATTTTCGATGTGAGTAGCACTTTTACATTTTTACAGTCATAATGTGAC
CAACTTGTAAAAAATGTTATGTTTTATGCCTATATTAGCCACCTAGTGGCTGCTCAT
TGCATAGCTTTTTCAGTTAACGTATAGCGCCATCTAGTGTATAACGTGTTTGTTGCA
ATTATAGATG 3'

FIGURE 2

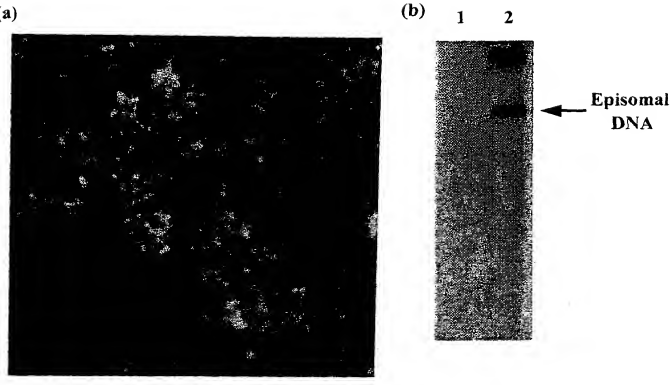

FIGURE 3

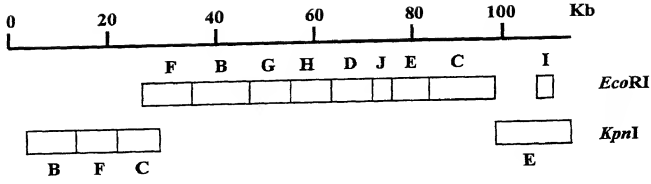

Figure 4

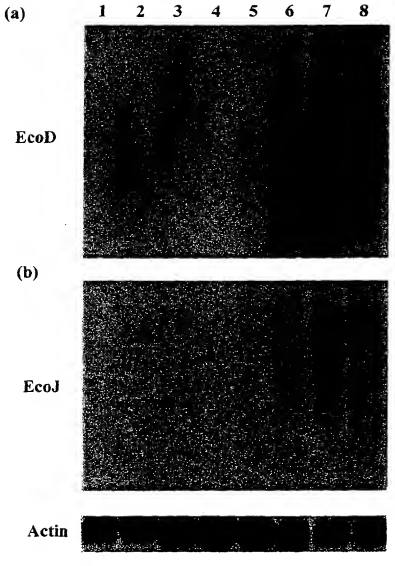

Figure 5

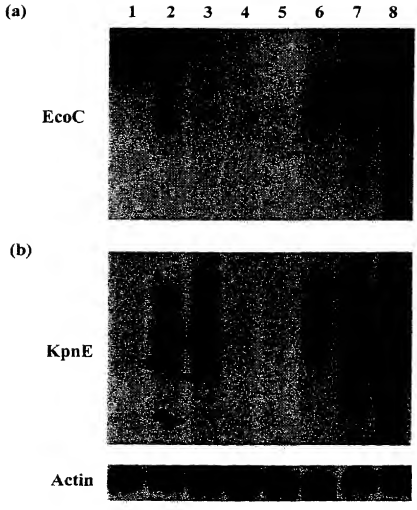

Figure 6

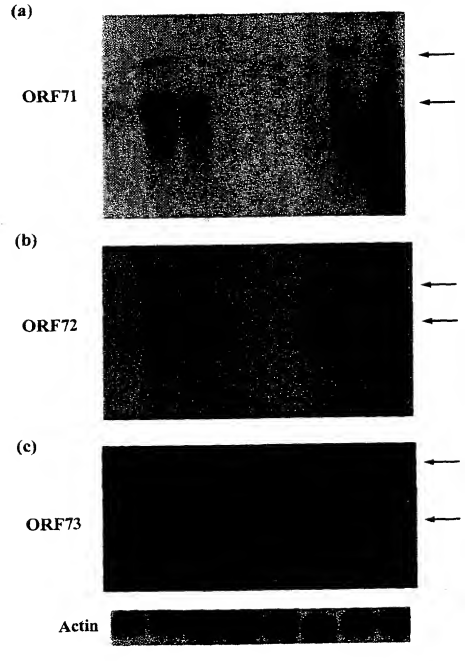

FIGURE 7

Table 1.

(ND = not determined; vw = very weak level of expression)

	Green-A549	Green Jurkat	Green MIAPACA	Green SW480
KpnB	vw	-	-	-
KpnC	vw	ND	ND	ND
KpnE	+	ND	ND	ND
KpnF	vw	-	-	-
EcoC	+	-	-	-
EcoD	vw	-	-	-
EcoE	vw	-	-	-
EcoF	vw	-	-	vw
EcoG	vw	-	-	-
EcoH	vw	-	-	-
EcoJ	vw	-	-	-

INTERNATIONAL SEARCH REPORT

Intern[illegible]onal Application No
PCT/GB 00/00537

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/86 C12N15/38 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NICHOLAS J. ET AL.: "Herpesvirus saimiri encodes homologues of G-protein-coupled receptors and cyclins." NATURE, vol. 355, 23 January 1992 (1992-01-23), pages 362-365, XP002138827 the whole document	1-5,21
A	WO 98 10083 A (MEREDITH DAVID MARK ;UNIV LEEDS (GB); MARKHAM ALEXANDER FRED (GB)) 12 March 1998 (1998-03-12) abstract	1-21
A	EP 0 362 732 A (BEHRINGWERKE AG) 11 April 1990 (1990-04-11) abstract	1-21

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 May 2000	14/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Galli, I

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter ional Application No
PCT/GB 00/00537

Patent document cited in search report	Publication date	Patent family member(s)	Publication dat
WO 9810083 A	12-03-1998	AU 4307197 A	26-03-1998
		BR 9711998 A	18-01-2000
		CN 1237209 A	01-12-1999
		CZ 9900697 A	11-08-1999
		EP 0939828 A	08-09-1999
EP 0362732 A	11-04-1990	DE 3834157 A	19-04-1990
		AU 615348 B	26-09-1991
		AU 4263989 A	12-04-1990
		CA 1340448 A	16-03-1999
		DK 494389 A	08-04-1990
		FI 894721 A	08-04-1990
		JP 2171190 A	02-07-1990
		KR 163170 B	16-11-1998
		PT 91903 A	30-04-1990
		US 6025153 A	15-02-2000

Claims

1 A nucleic acid comprising a nucleic acid sequence which encodes a promoter and hybridises under high stringency conditions to the nucleic acid sequence of SEQ ID NO:1, fragments and/or variants thereof.

2. A nucleic acid according to Claim 1 wherein the stringent conditions are 1 x SSC, 0.1% SDS at 65°C.

3. A nucleic acid according to either preceding claim wherein said promoter comprises a nucleic acid sequence of at least 329 bp and up to 2000 bp.

4. A nucleic acid according to Claim 3 wherein said promoter comprises a nucleic acid sequence of up to a length of 329, 630, 1000 or 1500 bp.

5. A recombinant DNA molecule containing at least one insert comprising the nucleic acid sequence of SEQ ID NO:1, or a fragment and/or variant thereof and encoding a promoter.

6. A gene therapy system comprising a vector which includes a nucleic acid comprising a nucleic acid sequence which encodes a promoter and hybridises under high stringency conditions to the nucleic acid sequence of SEQ ID NO:1, fragments and/or variants thereof, wherein said system is capable of driving heterologous gene expression during periods of latent infection by the vector in a target cell population.

7. A gene therapy system according to Claim 6 which further includes any one or more of the features of Claims 2-4.

8. A gene therapy system according to either of Claims 6 or 7 wherein the vector additionally comprises at least one therapeutic nucleic acid, whereby the promoter

encoded by SEQ ID NO:1 or fragment or variant thereof acts to drive expression of the therapeutic nucleic acid.

9. A gene therapy system according to any of Claims 6-8 wherein the vector is viral or non-viral.

10. Use of a gene therapy system according to any of Claims 6-9 for long term gene expression.

11. An HVS including a nucleic acid comprising a nucleic acid sequence which encodes a promoter and hybridises under high stringency conditions to the nucleic acid sequence of SEQ ID NO:1, fragments and/or variants thereof, the promoter acting in the latent state and the sequence encoding the promoter being positioned so as to drive expression of at least one therapeutic nucleic acid which has been inserted in the HVS.

12. An HVS according to Claim 11 and further including any of the features of Claims 2-4, 8 or 10.

13. A method of manufacturing an expression vector comprising a promoter according to any of Claims 1-4, or a gene therapy system according to any of Claims 6-10 or a HVS vector according to either Claim 11 or 12, the method comprising transfecting a cell with a nucleic acid sequence encoding the promoter of SEQ ID NO:1, or fragment or variant thereof or a nucleic acid sequence which hybridises under high stringency conditions to the sequence of SEQ ID NO:1 or any part thereof.

14. A method according to Claim 13 which further comprises the steps of selecting siad nucleic acid sequence and amplifying it and subsequently purifying it prior to transfecting a cell population.

15. A method of treatment comprising administering a therapeutically effective amount of a promoter according to any of Claims 1-4 or a gene therapy system according to any of Claims 6-10 or a HVS vector according to either Claim 11 or 12, to an individual requiring treatment.

16. A promoter according to any of Claims 1-4 or a gene therapy system according to any of Claims 6-10 or a HVS vector according to either Claim 11 or 12, for use as a pharmaceutical.

17. A pharmaceutical composition comprising a promoter according to any of Claims 1-4 or a gene therapy system according to any of Claims 6-10 or a HVS vector according to either Claim 11 or 12, and a pharmaceutically acceptable excipient, diluent or carrier.

18. A pharmaceutical composition according to Claim 17 that is formulated as a nasal spray, or for injection or for oral/parenteral administration.

19. Use of a promoter according to any of Claims 1-4 or a gene therapy system according to any of Claims 6-10 or a HVS vector according to either Claim 11 or 12, in the manufacture of a medicament for treating cancer.

20. Use of a promoter according to any of Claims 1-4 or a gene therapy system according to any of Claims 6-10 or a HVS vector according to either Claim 11 or 12, in the manufacture of a medicament for treating degenerative disorders.

21. An isolated nucleic acid encoding a promoter, the nucleic acid being selected from the group consisting of:

(a) DNA having the nucleotide sequence given herein as SEQ ID NO:1 and which encodes the promoter; and

(b) nucleic acids which hybridize to DNA of (a) above (e.g., under stringent conditions) and which encode the promoter.

AMENDED CLAIMS

1 An isolated nucleic acid comprising the nucleic acid sequence as set forth in SEQ ID NO:1 and which encodes a latency promoter, the nucleic acid consisting of a sequence positioned upstream of the initiation codon of ORF 73 of HVS or comprising a nucleic acid sequence which has at least 75% homology with SEQ ID NO:1 and which encodes a latency promoter.

2. A nucleic acid according to Claim 1 wherein the nucleic acid sequence which has at least 75% homology with SEQ ID NO:1 hybridises under stringent conditions of 1 x SSC, 0.1% SDS at 65°C to the sequence as set forth in SEQ ID NO:1.

3. A nucleic acid according to either preceding claim wherein said latency promoter is encoded by a nucleic acid sequence of at least 329 bp and up to 2000 bp.

4. A nucleic acid according to Claim 3 wherein said latency promoter is encoded by a nucleic acid sequence of up to a length of 329, 630, 1000 or 1500 bp.

5. Use of a nucleic acid sequence as set forth in SEQ ID NO:1, the nucleic acid consisting of a sequence positioned upstream of the initiation codon of ORF 73 of HVS or comprising a nucleic acid sequence which has at least 75% homology with SEQ ID NO:1 as a latency promoter.

6. Use according to claim 5 further including any one or more of the features of claims 2 to 4.

7. A recombinant DNA molecule containing at least one insert comprising the nucleic acid sequence as set forth in SEQ ID NO:1 and which encodes a latency promoter, the nucleic acid consisting of a sequence positioned upstream of the initiation codon of ORF 73 of HVS or comprising a nucleic acid sequence which has at least 75% homology with SEQ ID NO:1 and which encodes a latency promoter.

8. A recombinant DNA molecule according to claim 7 further including any one or more of the features of claims 2 to 4.

9. A gene therapy system comprising a vector which includes a nucleic acid sequence as set forth in SEQ ID NO:1 and which encodes a latency promoter, the nucleic acid consisting of a sequence positioned upstream of the initiation codon of ORF 73 of HVS or comprising a nucleic acid sequence which has at least 75% homology with SEQ ID NO:1. and which encodes a latency promoter, wherein said system is capable of driving heterologous gene expression during periods of latent infection by the vector in a target cell population.

10. A gene therapy system according to claim 9 further including any one or more of the features of claims 2 to 4.

11. A gene therapy system according to either Claim 9 or 10 wherein the vector additionally comprises at least one therapeutic nucleic acid, expression of which is driven by the latency promoter.

12. A gene therapy system according to any one of Claims 9 to 11 wherein the vector is viral or non-viral.

13. Use of a gene therapy system according to any one of Claims 9 to 12 for long term gene expression.

14. An HVS including the nucleic acid sequence nucleic acid sequence as set forth in SEQ ID NO:1 and which encodes a latency promoter, the nucleic acid consisting of a sequence positioned upstream of the initiation codon of ORF 73 of HVS or comprising a nucleic acid sequence which has at least 75% homology with SEQ ID NO:1. and which encodes a latency promoter, the latency promoter acting in the latent state and the sequence encoding the latency promoter being positioned so

as to drive expression of at least one therapeutic nucleic acid which has been inserted in the HVS.

15. An HVS according to claim 14 and further including any one or more of the features of claims 1 to 4, 11 or 13.

16. A method of manufacturing an expression vector comprising a nucleic acid according to any one of Claims 1 to 4, or a gene therapy system according to any of Claims 9 to 12 or a HVS vector according to either Claim 14 or 15, the method comprising transfecting a cell with the nucleic acid sequence encoding the latency promoter.

17. A method according to Claim 16 which further comprises the steps of selecting said nucleic acid sequence and amplifying it and subsequently purifying it prior to transfecting a cell population.

18. A method of treatment comprising administering a therapeutically effective amount of a nucleic acid according to any one of Claims 1 to 4 or a gene therapy system according to any of Claims 9 to 12 or a HVS vector according to either Claim 14 or 15, to an individual requiring treatment.

19. A nucleic acid according to any one of Claims 1 to 4 or a gene therapy system according to any of Claims 9 to 12 or a HVS vector according to either Claim 14 or 15, for use as a pharmaceutical.

20. A pharmaceutical composition comprising the nucleic acid according to any one of Claims 1 to 4 or a gene therapy system according to any of Claims 9 to 12 or a HVS vector according to either Claim 14 or 15, and a pharmaceutically acceptable excipient, diluent or carrier.

21. A pharmaceutical composition according to Claim 20 that is formulated as a nasal spray, or for injection or for oral/parenteral administration.

22. Use of a latency promoter encoded by the nucleic acid sequence according to any one of Claims 1 to 4 or a gene therapy system according to any one of Claims 9 to 12 or a HVS vector according to either Claim 14 or 15, for the manufacture of a medicament for treating cancer.

23. Use of a latency promoter encoded by the nucleic acid sequence according to any one of Claims 1 to 4 or a gene therapy system according to any one of Claims 9 to 12 or a HVS vector according to either Claim 14 or 15, for the manufacture of a medicament for treating degenerative disorders.

24. An isolated nucleic acid encoding a latency promoter, the nucleic acid being selected from the group consisting of:

(a) DNA having the nucleotide sequence given herein as SEQ ID NO:1 or fragment thereof and which encodes the latency promoter; and

(b) nucleic acids which hybridize to DNA of (a) above (e.g., under stringent conditions) and which encode the latency promoter.

Latency-associated regulatory region
(Initiation codon of ORF 73 shown in bold)

5' ACCCAGAGAGCTGGACACTAGAACTAGAACCTAATGCATCAAAGCATTATGAATC
TTTATGGCTCAACTTTCACGTTCCTCAAACTACTAAAAGCATTATATTACAAGCCCT
TCGTGGCACAATTTTCCAGGATGGCTTGTGGCAAGTACTTGGACTGAGATACAAACA
CGATGCTCAAGAATATATTATGCAACAAAATGGAACAATTGCAATGAGTTATCATAG
TGCTAAGATAAATCCTTACTTGTATGCAATGCATTATCCAAGGAACCCCTCTGGCAA
TTCATCTGTAGCTGGCATATGTTCAAAGAATGGCAGGCATCTTGCGTTGCTTGTAGA
ACCAGCCCTTTCTTTTCATACTTGGCAATGGCAACATATACCTAAACCTCTAGTAAC
TTCTCCATGGGCATTAATGTATCAATGTATGTTCTTGTGGTGTGTAAAAGAATGATT
GTACTAAGGAACAGTAATAAAAACTCTGACACTAAGATACGATAATATAACTATTTA
TTTATCAAGTGAGCCGCTCTACACTCTAACAGTGACAAATAGTTTTACACCATGCAG
CCATGCGCTGCCTAAAGAGACTTCCAAACATAGCAAACATCAGAGGTAACATACAAT
AATATAGTACCAACAGCATATATGTACATTGAATTCCATACACTATAGCAGATCTCT
TTGCACATGTCTCTTCTATTACACCAACACGCAACAAAGTATCAATGCTTTCCATAA
TATAGTATGGTATACAAAACACTATGAATAGCAGTGTTGTCATTGTAATTATCGTGA
CTACCTCTGCTCTTTTAGACAGCTTTGTCTTGAATAACTTATAACATGACATACTAT
AGCATATTACAGTAATAAAGAGGGGTCCTGCAAAGCTATACCATGTGTGAAAAGTGT
TTAGCTTTGTGCGTAGCTGCTCAGTCAACACACCATCCTCCTCTATGCAAGAAGATG
GTTCATAATATGATGTCACCATCACATGAGGAAGTGCTCCAAAGCAGGCTAATACAA
ATGAACAGCACAGAAATACTTGCCCAATAAGAGTCTTTTTCACCCACAGTCTAGTAG
CACAAAATATTAGCAGACAACGCAAGACACTAATAAAAACTAATATGAAAGGAGACC
AATAAATGCTGAGATTTAAGAAAAAAGCTTCCAGCTTACACAGCTCAGTATTCATAA
AAATTTCAAACATGCGCAAAAGTCTCATTAGCAGATACCCAGCTAAGAACAAGCTGT
TGAGACAAAATCCCATCATCAAGTAGTCAAAACTTTGAGCTTGAGCTCTATACTTTA
GAAAAGTCCTCAGTACAAGAGAATTCCCAATTGCATTGCATAAAAAACATCAACACAT
ATATGAATGCTAGTGCACTCTCTGAAATTAAAAAGTTCACTACACACGGCGCTACAT
CTCCATAATATATGTCTCCACTATAATTGTAAGAATAGTTGCTAAAGTCTTCACTAC
TGAAGTCCAGCTTGACCTCCATAGCGAACTACAAAATAAATTTATATAAATTATTCA
CCCAATAACTTGAAATTTAAAGAATTAGGACAAAAGAATGTATATCCTACCTTTCTT
TGCAGCCTGACAGCAAGCTACTGAAAAAGTTACTTTTTATTTTGTTTTAGTAGCTAG
GTGTGGTTTTACATATGTTTTGTGGCTACACAGTAGATTTAACAAATAGCCACGCCC
CCTACGCTACGTCTAAGGAGGAGCTTAATTCCAAACGAGTGGCGGGATTTCGCTAAA
GTCACTGAAGAACTTGCATCTTAATTCATCCGCGGCTGCAACCTTCAAACAAAAAAG
GAGGTTTCGATTTTCGATGTGAGTAGCACTTTTACATTTTTACAGTCATAATGTGAC
CAACTTGTAAAAAATGTTATGTTTTATGCCTATATTAGCCACCTAGTGGCTGCTCAT
TGCATAGCTTTTTCAGTTAACGTATAGCGCCATCTAGTGTATAACGTGTTTGTTGCA
ATTATAG**ATG** 3'

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Harrison Goddard Foote
Tower House
Merrion Way
Leeds LS2 8PA
GRANDE BRETAGNE

08. JUN. 2001 *056291

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	05.06.2001

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
LPB/P32042WO	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/00537	18/02/2000	19/02/1999

Applicant
UNIVERSITY OF LEEDS et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	CLEERE, C Tel.+49 89 2399-8061

Form PCT/IPEA/416 (July 1992)

TENT COOPERATION TR Y

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
LPB/P32042WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/00537	18/02/2000	19/02/1999
International Patent Classification (IPC) or national classification and IPC C12N15/86		
Applicant UNIVERSITY OF LEEDS et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05/09/2000	05.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer SCHEFFZYK, I Telephone No. +49 89 2399 8602

Form PCT/IPEA/409 (cover sheet) (January 1994)

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-23	as originally filed	

Claims, No.:

1-24	with telefax of	08/05/2001

Drawings, sheets:

1/8-8/8	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☐ claims Nos. .

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☒ the claims, or said claims Nos. 1,7,9,14 (see section VII/2). are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims

	No:	Claims	1-6,8,10-13,15-24
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-6,8,10-13,15-24
Industrial applicability (IA)	Yes:	Claims	1-12,14-17,19-24
	No:	Claims	13 and 18: see section VIII/4).

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

SECTION V----------

The subject-matter of present claims is not considered to be clearly delimitated from the sequence taught in Nature, vol. 355, 23.01.92, pp. 362-365, Nicholas J. et al.(1), since the sequence taught in (1) is the complement sequence of the sequence shown in SEQ.ID. NO.1 and is thus considered to fall within the scope of claim 2. In addition, with respect to claim 24(b) it is noted that in the absence of an indication concerning the hybridization conditions (the stringent conditions mentioned in claim 24(b) are only facultative) the subject-matter of said claim covers any readily available nucleic acid sequence.
Moreover, it is noted that due to the wording of present claims (comprising) the complete HSV genome is covered by claims which are directed to nucleic acid molecules.
Thus, claims 2-6,8,10-13, 15-24 do not meet the requirements of Art. 33(2)(3) PCT.

Remark: Claims clearly limited to nucleic acids consisting of the sequence shown in SEQ.ID.NO. 1 basically would be deemed novel and inventive.

SECTION VII----------

1). For the subject-matter of newly-filed claim 8 no basis can be found in the application as filed (see original claim 5 which only is directed to a DNA molecule containing at least one insert comprising the sequence shown in SEQ.ID.NO.1) (Art. 34(2)(b) PCT).

2). The subject-matter of newly-filed claims 1, 7, 9, 14 also extends beyond the content of the application as filed since according to the content of the application as filed the sequence having at least 75% homology with the sequence shown in SEQ.ID.NO. 1 must hybridise to the sequence shown in SEQ.ID.NO. 1. In the absence of any basis for these claims novelty and inventive of these claims have not been assessed.

3). Claim 24 is deemed superfluous in view of claim 1.

4). From the description it appears that the sequence hybridising to the sequence shown in SE.ID.NO. 1 encodes the promotor but not the sequence shown in SEQ.ID.NO. 1 itself?! (see e.g. page 4, 3rd paragraph). Correspondingly, some of the claims are not in line with the specification.

SECTION VIII----------

1). The scope of claims using simultaneously the terms "consists of" and "contains/comprise" is ambiguous (see e.g. claim 1) (see also for instance claims 3 and 4 referring to claim 1, which are also contrary to the meaning of "consist of" used in claim 1) (Art. 6 PCT).

2). Concerning claim 24(b) it is noted that a nucleic acid which hybridizes to the encoding sequence shown in SEQ.Id.NO. 1 obviously cannot be also encoding.

3). Claims 5 and 6 are incomplete (use for what?).

4). The wording of claims 6 and 8 is unclear.

5). Claims 18, 22 and 23 are not supported by the specification since present application fails to show facts and data which would be suitable to demonstrate that the sequence shown in SEQ.ID. NO. 1 is actually useful for medical treatments as defined in these claims.

6). Claims 13 and 18 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of this claim (Article 34(4)(a)(i) PCT).

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
LPB/P32042WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/00537	18/02/2000	19/02/1999

International Patent Classification (IPC) or national classification and IPC
C12N15/86

Applicant
UNIVERSITY OF LEEDS et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05/09/2000	05.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer SCHEFFZYK, I Telephone No. +49 89 2399 8602

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/00537

I. Basis f th report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-23	as originally filed	

Claims, No.:

1-24	with telefax of	08/05/2001

Drawings, sheets:

1/8-8/8	as originally filed

2. With regard to the **language,** all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☐ claims Nos. .

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☒ the claims, or said claims Nos. 1,7,9,14 (see section VII/2). are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims

	No:	Claims	1-6,8,10-13,15-24
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-6,8,10-13,15-24
Industrial applicability (IA)	Yes:	Claims	1-12,14-17,19-24
	No:	Claims	13 and 18: see section VIII/4).

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
se separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

SECTION V----------

The subject-matter of present claims is not considered to be clearly delimitated from the sequence taught in Nature, vol. 355, 23.01.92, pp. 362-365, Nicholas J. et al.(1), since the sequence taught in (1) is the complement sequence of the sequence shown in SEQ.ID. NO.1 and is thus considered to fall within the scope of claim 2. In addition, with respect to claim 24(b) it is noted that in the absence of an indication concerning the hybridization conditions (the stringent conditions mentioned in claim 24(b) are only facultative) the subject-matter of said claim covers any readily available nucleic acid sequence.
Moreover, it is noted that due to the wording of present claims (comprising) the complete HSV genome is covered by claims which are directed to nucleic acid molecules.
Thus, claims 2-6,8,10-13, 15-24 do not meet the requirements of Art. 33(2)(3) PCT.

Remark: Claims clearly limited to nucleic acids consisting of the sequence shown in SEQ.ID.NO. 1 basically would be deemed novel and inventive.

SECTION VII----------

1). For the subject-matter of newly-filed claim 8 no basis can be found in the application as filed (see original claim 5 which only is directed to a DNA molecule containing at least one insert comprising the sequence shown in SEQ.ID.NO.1) (Art. 34(2)(b) PCT).

2). The subject-matter of newly-filed claims 1, 7, 9, 14 also extends beyond the content of the application as filed since according to the content of the application as filed the sequence having at least 75% homology with the sequence shown in SEQ.ID.NO. 1 must hybridise to the sequence shown in SEQ.ID.NO. 1. In the absence of any basis for these claims novelty and inventive of these claims have not been assessed.

3). Claim 24 is deemed superfluous in view of claim 1.

4). From the description it appears that the sequence hybridising to the sequence shown in SE.ID.NO. 1 encodes the promotor but not the sequence shown in SEQ.ID.NO. 1 itself?! (see e.g. page 4, 3rd paragraph). Correspondingly, some of the claims are not in line with the specification.

SECTION VIII----------

1). The scope of claims using simultaneously the terms "consists of" and "contains/comprise" is ambiguous (see e.g. claim 1) (see also for instance claims 3 and 4 referring to claim 1, which are also contrary to the meaning of "consist of" used in claim 1) (Art. 6 PCT).

2). Concerning claim 24(b) it is noted that a nucleic acid which hybridizes to the encoding sequence shown in SEQ.Id.NO. 1 obviously cannot be also encoding.

3). Claims 5 and 6 are incomplete (use for what?).

4). The wording of claims 6 and 8 is unclear.

5). Claims 18, 22 and 23 are not supported by the specification since present application fails to show facts and data which would be suitable to demonstrate that the sequence shown in SEQ.ID. NO. 1 is actually useful for medical treatments as defined in these claims.

6). Claims 13 and 18 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of this claim (Article 34(4)(a)(i) PCT).

AMENDED CLAIMS

1 An isolated nucleic acid comprising the nucleic acid sequence as set forth in SEQ ID NO:1 and which encodes a latency promoter, the nucleic acid consisting of a sequence positioned upstream of the initiation codon of ORF 73 of HVS or comprising a nucleic acid sequence which has at least 75% homology with SEQ ID NO:1 and which encodes a latency promoter.

2. A nucleic acid according to Claim 1 wherein the nucleic acid sequence which has at least 75% homology with SEQ ID NO:1 hybridises under stringent conditions of 1 x SSC, 0.1% SDS at 65°C to the sequence as set forth in SEQ ID NO:1.

3. A nucleic acid according to either preceding claim wherein said latency promoter is encoded by a nucleic acid sequence of at least 329 bp and up to 2000 bp.

4. A nucleic acid according to Claim 3 wherein said latency promoter is encoded by a nucleic acid sequence of up to a length of 329, 630, 1000 or 1500 bp.

5. Use of a nucleic acid sequence as set forth in SEQ ID NO:1, the nucleic acid consisting of a sequence positioned upstream of the initiation codon of ORF 73 of HVS or comprising a nucleic acid sequence which has at least 75% homology with SEQ ID NO:1 as a latency promoter.

6. Use according to claim 5 further including any one or more of the features of claims 2 to 4.

7. A recombinant DNA molecule containing at least one insert comprising the nucleic acid sequence as set forth in SEQ ID NO:1 and which encodes a latency promoter, the nucleic acid consisting of a sequence positioned upstream of the initiation codon of ORF 73 of HVS or comprising a nucleic acid sequence which has at least 75% homology with SEQ ID NO:1 and which encodes a latency promoter.

8. A recombinant DNA molecule according to claim 7 further including any one or more of the features of claims 2 to 4.

9. A gene therapy system comprising a vector which includes a nucleic acid sequence as set forth in SEQ ID NO:1 and which encodes a latency promoter, the nucleic acid consisting of a sequence positioned upstream of the initiation codon of ORF 73 of HVS or comprising a nucleic acid sequence which has at least 75% homology with SEQ ID NO:1. and which encodes a latency promoter, wherein said system is capable of driving heterologous gene expression during periods of latent infection by the vector in a target cell population.

10. A gene therapy system according to claim 9 further including any one or more of the features of claims 2 to 4.

11. A gene therapy system according to either Claim 9 or 10 wherein the vector additionally comprises at least one therapeutic nucleic acid, expression of which is driven by the latency promoter.

12. A gene therapy system according to any one of Claims 9 to 11 wherein the vector is viral or non-viral.

13. Use of a gene therapy system according to any one of Claims 9 to 12 for long term gene expression.

14. An HVS including the nucleic acid sequence nucleic acid sequence as set forth in SEQ ID NO:1 and which encodes a latency promoter, the nucleic acid consisting of a sequence positioned upstream of the initiation codon of ORF 73 of HVS or comprising a nucleic acid sequence which has at least 75% homology with SEQ ID NO:1. and which encodes a latency promoter, the latency promoter acting in the latent state and the sequence encoding the latency promoter being positioned so

as to drive expression of at least one therapeutic nucleic acid which has been inserted in the HVS.

15. An HVS according to claim 14 and further including any one or more of the features of claims 1 to 4, 11 or 13.

16. A method of manufacturing an expression vector comprising a nucleic acid according to any one of Claims 1 to 4, or a gene therapy system according to any of Claims 9 to 12 or a HVS vector according to either Claim 14 or 15, the method comprising transfecting a cell with the nucleic acid sequence encoding the latency promoter.

17. A method according to Claim 16 which further comprises the steps of selecting said nucleic acid sequence and amplifying it and subsequently purifying it prior to transfecting a cell population.

18. A method of treatment comprising administering a therapeutically effective amount of a nucleic acid according to any one of Claims 1 to 4 or a gene therapy system according to any of Claims 9 to 12 or a HVS vector according to either Claim 14 or 15, to an individual requiring treatment.

19. A nucleic acid according to any one of Claims 1 to 4 or a gene therapy system according to any of Claims 9 to 12 or a HVS vector according to either Claim 14 or 15, for use as a pharmaceutical.

20. A pharmaceutical composition comprising the nucleic acid according to any one of Claims 1 to 4 or a gene therapy system according to any of Claims 9 to 12 or a HVS vector according to either Claim 14 or 15, and a pharmaceutically acceptable excipient, diluent or carrier.

21. A pharmaceutical composition according to Claim 20 that is formulated as a nasal spray, or for injection or for oral/parenteral administration.

22. Use of a latency promoter encoded by the nucleic acid sequence according to any one of Claims 1 to 4 or a gene therapy system according to any one of Claims 9 to 12 or a HVS vector according to either Claim 14 or 15, for the manufacture of a medicament for treating cancer.

23. Use of a latency promoter encoded by the nucleic acid sequence according to any one of Claims 1 to 4 or a gene therapy system according to any one of Claims 9 to 12 or a HVS vector according to either Claim 14 or 15, for the manufacture of a medicament for treating degenerative disorders.

24. An isolated nucleic acid encoding a latency promoter, the nucleic acid being selected from the group consisting of:

(a) DNA having the nucleotide sequence given herein as SEQ ID NO:1 or fragment thereof and which encodes the latency promoter; and

(b) nucleic acids which hybridize to DNA of (a) above (e.g., under stringent conditions) and which encode the latency promoter.

P32042wo1.amendedclaims

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
LPB/P32042WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/ 00537	18/02/2000	19/02/1999
Applicant		
UNIVERSITY OF LEEDS et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   LATENCY-ASSOCIATED REGULATORY REGION FROM HERPESVIRUS SAIMIRI (HVS)

5. With regard to the **abstract,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. –

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

ational application No.
PCT/GB 00/00537

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

R mark n Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

Line 2: after word "therapy" insert "derived from the Herpesvirus saimiri (HVS) ORF73 (ECLFI) gene."

INTERNATIONAL SEARCH REPORT

Int[redacted]al Application No
PCT/GB 00/00537

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/86 C12N15/38 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NICHOLAS J. ET AL.: "Herpesvirus saimiri encodes homologues of G-protein-coupled receptors and cyclins." NATURE, vol. 355, 23 January 1992 (1992-01-23), pages 362-365, XP002138827 the whole document	1-5,21
A	WO 98 10083 A (MEREDITH DAVID MARK ;UNIV LEEDS (GB); MARKHAM ALEXANDER FRED (GB)) 12 March 1998 (1998-03-12) abstract	1-21
A	EP 0 362 732 A (BEHRINGWERKE AG) 11 April 1990 (1990-04-11) abstract	1-21

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 May 2000	14/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Galli, I

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[redacted]nal Application No
PCT/GB 00/00537

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9810083 A	12-03-1998	AU 4307197 A	26-03-1998
		BR 9711998 A	18-01-2000
		CN 1237209 A	01-12-1999
		CZ 9900697 A	11-08-1999
		EP 0939828 A	08-09-1999
EP 0362732 A	11-04-1990	DE 3834157 A	19-04-1990
		AU 615348 B	26-09-1991
		AU 4263989 A	12-04-1990
		CA 1340448 A	16-03-1999
		DK 494389 A	08-04-1990
		FI 894721 A	08-04-1990
		JP 2171190 A	02-07-1990
		KR 163170 B	16-11-1998
		PT 91903 A	30-04-1990
		US 6025153 A	15-02-2000